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Sanitary conditions of the Goliath grouper Epinephelus itajara in captivity on estuarine environment: case report

[*Condições sanitárias do mero Epinephelus itajara em cativeiro em ambiente estuarino: relato de caso*]

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ABSTRACT

This study aimed to report the sanitary conditions through the hematological analysis of grouper E. itajara reared in captivity on estuarine conditions. Seven Goliath groupers (1,881.5±1,246.03g) were captured and kept in two tanks located on estuary. After 20 days, fish were collected for morphologic and hemato-physiologic evaluation. Two fish had clinical signs such as hemorrhagic spots and loss of scale due to agonistic behavior. Blood samples were collected, and the hematological parameters (biochemical, erythrogram and leukogram) were determined. Blood cells were characterized by their size, color and shape. Univariate statistic and principal components analysis were used to identify a hematological standard between fish with or without clinical signs. Four leukocyte types were found: lymphocyte, monocyte, neutrophil and basophil. Regardless of the clinical signs the cell morphology did not present any difference among the fish. However, there is a significant correlation between erythrocyte and lactate on fish with clinical signs. Thus, agonistics encountered among the fish is a stressing factor in captivity conditions making it necessary to have adequate management related to the size of fish and stocking density.

Keywords: goliath grouper, blood cell, hematology, mero, characterization

RESUMO

Este estudo avaliou as condições sanitárias do peixe mero E. itajara *mantido sob cativeiro, em condições estuarinas, pelas análises hematológica e morfológica. Sete peixes (1.881,5±1.246,03g) foram capturados e mantidos em tanques localizados no estuário. Após 20 dias, os peixes foram coletados para avaliações morfológica e hematológica. Dois peixes tiveram sinais clínicos, como manchas hemorrágicas e perda de escamas devido ao comportamento agonístico entre os peixes. Amostras sanguíneas foram coletadas dos peixes anestesiados com auxílios de seringas umedecidas com EDTA 3%. Determinaram-se os parâmetros hematológicos (bioquímico, eritrograma e leucograma). Células sanguíneas foram caracterizadas por seu tamanho, cor e padrão. Estatística univariada e análises de componentes principais foram usadas para identificar um padrão hematológico entre os peixes com e sem sinais clínicos. Quatro tipos de leucócitos foram encontrados: linfócito, monócito, neutrófilo e basófilo. Os sinais clínicos não apresentaram diferença entre os peixes e a morfologia celular. Contudo, observou-se uma correlação entre os peixes com sinais clínicos e a quantidade de eritrócitos e lactato. Assim, encontros antagônicos entre os peixes são um fator estressante em condições de cativeiro, tornando-se necessário um manejo mais adequado relacionado ao tamanho dos indivíduos e à densidade de estocagem.*

Palavras-chave: garoupa, célula sanguínea, hematologia, mero, caracterização

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INTRODUCTION

The Goliath grouper *Epinephelus itajara* (Lichtenstein, 1822) is the largest grouper in the Atlantic Ocean, exceeding 2.5m in total length and 350kg in weight (Bullock *et al*., 1992). The adult specimens are distributed throughout the Atlantic Ocean with a long life cycle, late reproduction and pacific behavior making these species susceptible to the predatory fishing (Bullock *et al*.,1992; Craig and Hasting, 2007). In worldwide terms, the IUCN classifies this species as critically endangered.

In Brazil, the fish population declined by the degradation of their mangrove habitat and missing ecological data as well as the lack of knowledge about captive measures for this species. For that, a regulation was enacted from 2002 to 2015 which forbids fishing of Goliath groupers *E. itajara* and turned this species in to the first marine fish species to have an individual regulation in Brazilian waters (Silva *et al*., 2014). Efforts for species preservation have been carried out such as the project "Meros do Brasil". Actions to understand and to increase the knowledge about the species concerning preservation is important, and the captivity production of Goliath grouper can be one of these alternatives (Sanches *et al*., 2015).

The captivity process is stressful for wild fish and the adaption process to a new habitat should attempt to mimic the biological and ecological requirements of the species. In that process, the fish modify hemato-physiological patterns to adapt to the captivity condition (Selye, 1950). The use of hematology could be a tool to evaluate the sanitary conditions and to understand the adaptation mechanism. In addition, it is important to know the morphological aspects and the baseline values of the hematological profile to evaluate the alterations in the blood (Paixão *et al*., 2017).

However, studies about natural hematological profile of marine fish, mainly for groupers (Fogliarine *et al*., 2017) is still missing. This biologic information improves the rearing technology for adjusting the captivity conditions to species requirement. For these reasons, the aim of this study was to evaluate the sanitary conditions of the Goliath grouper *Epinephelus itajara* on captivity conditions, through the hematological changes.

CASUISTRY

Seven Goliath groupers (*E. itajara*, 1.881,5±1.246,03g) were collected with the aid of fishermen (ethic committee 006/2018, SISBIO 62451-1, SISGEn A775B31) and stocked in two 200L polyethylene tanks, into an estuarine river, during 20 days. The tanks were perforated to allow the exchange of water and to avoid fish escape.

Two fish from the same tank presented clinical signs such as hemorrhagic skin and scale loss (Figure 1). Sterile swabs were used on the wounds for bacterial analysis. That fish presented the highest values of weight (3.5 and 3.3kg). The other five fish presented normal conditions. The fish were anesthetized in eugenol bath (60mg.L^{-1}) and blood samples were collected by caudal vein puncture using syringes with EDTA (3%). Biochemical parameters such as glucose (mg.dL^{-1}), triglyceride (g.dL^{-1}), cholesterol (g.dL^{-1}) and lactate (g.dL^{-1}) were determined using an automatic measurer (Accuchek Active®) and total plasmatic protein (TPP, g.dL-^{1}) using a refractometer.

Figure 1. Hemorragic skin and scale loss from two *E. itajara* fish observed after 20 days in captivity conditions.

The hemoglobin (g.dL^{-1}) was determined using a biochemical analyzer, the hematocrit (%) was measured by the micro hematocrit method (Goldenfarb *et al*., 1971) and the total counting of erythrocyte (µL) was in Neubauer chamber using two different concentrations of saline solution (NaCl 0.65 and 0.85%). Hematimetric index such as mean corpuscular volume (MCV), mean hemoglobin concentration (MHC) and mean corpuscular hemoglobin concentration (MCHC) were calculated according Vallada, (1999).

Leukocytes (white blood cells) were counting using the panchromic coloration according to to Rosenfeld (1947). Blood white and red cells were characterized by their size, color and shape. Microscopic *bio line SSI tech research* (Bel engineering) equipped by camera (Bel photonics) was used to take the blood cell pictures (Blue filter, coloration red 1.92 green 0.98 blue 1.25, brightness 25, contrast 1.62, saturation 75, image quality 1860x1200 pixel, scale 8.26 µm). Cell length and width, nuclear length and width and cell area were determined. Cell shapes (fusiform, spindle, ovoid, round and warped) and colors (blue, gray, purple and pink) were also reported.

The water quality parameters were measured with a multi-parameter analyzer (Hanna HI98196) for temperature (27,7ºC), dissolved oxygen (6,22 mg.L^{-1}), conductivity (4,1 mS.cm^{-1}). Salinity (20) and pH (7.56) were measured with refractometer and ATC Pocket Pencil, respectively.

Hematological data were submitted to the normality test by Shapiro. Blood data comparing the fish with and without clinical signs was analyzed by t-test ($P<0.05$) and the multivariate statistic of principal components analysis (PCA) was performed to understand the relationship between the hematological parameters and the fish conditions.

The fish with clinical signs presented increase in plasmatic lactate (Table 1) and reduction in erythrocyte count (Table 2). Other biochemical and hematological parameters had no difference. That fish showed an opportunistic bacterial infection, identified with the aid of a mass spectrophotometer (MALDI-TOF method according to Croxatto *et al*., 2012) as *Vibrio parahaemolyticus*.

Table 1. Mean values ± standard (g.dL^{-1}) deviation of biochemical parameters for *E. itajara* presenting clinical signs and no signs

	Glucose	Triglycerides	Cholesterol	Lactate	TPP
Clinical Signs	21.5±7.77	119.5±75.6	177.00±32.5	3.45±1.34a	5.6±0.70
No signs	40±22.31	102.4±48.7	187.4±21.12	1.96±0.39b	6.43±0.41

TPP=Total plasmatic protein

Table 2. Mean values ± standard deviation of hematological parameters of *E. itajara*

	Red Blood Cell			White Blood Cell	
	Clinical signs	No clinical signs		Clinical signs	No clinical signs
Ery (x10^6)	1.5±0.4a	0.9±0.13b*	Leu (x10^3)	68±21.9	63.7±47.3
Ery (x10^6)		1.13±0.42$^{\#}$	Thro (x10^3)	32.5±24.0	38.1±8.5
Hemat (%)	26.8±8.8	35.5±5.1	Lym (x10^3)	56.2±3.8	64.1±41.1
Hemoglo	5.8±1.5	7.0±1.8	Mon (x10^3)	12.3±8.0	8.77±7.6
MVC (fL)	183.7±101.8	400.3±109.2	Neu (x10^3)	4.1±3.1	2.25±1.89
MHC (pg)	35.4±18.6	79.3±24.8	Eos (x10^3)	ND	ND
MCHC (%)	19.4±0.6	20.6±7.5	Bas (x10^3)	0	0.13

*Saline solution NaCl 0.65%, # Saline solution NaCl 0.85%, Ery-Erythrocyte, Thro-Thrombocyte, Lym-Lymphocyte, Mon-Monocyte, Neut-Neutrophil, Eos-Eosinophil, Bas-Basophil, mean corpuscular volume (CMV), Mean Hemoglobin concentration (CHM) and Mean Corpuscular Hemoglobin Concentration (CHCM), ND (not determined).

Most erythrocytes were ovoid, but some cells had round form. They had basophilic cytoplasm showing gray color and round nucleus. Thrombocytes showed fusiform and spindle. Monocyte showed the presence of vacuoles. Only neutrophils have bilobed nuclear and small granules in the cytoplasm (Figure 2).

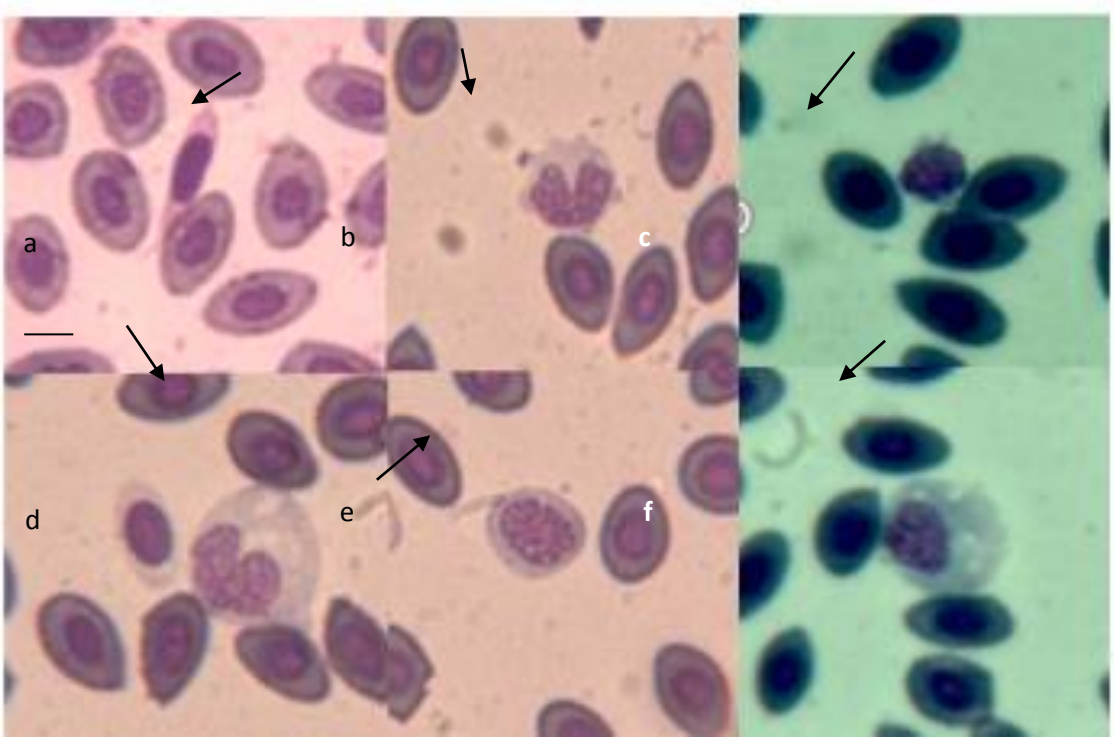

Figure 2. Cell blood from *E. itajara* in microscopic image: Thrombocyte (a), Neutrophil (b), Lymphocyte (c), (d) Monocyte, Immature leukocyte (e), Monocyte (f), Scale 8,26µm (-).

The blood cell sizes varied between 10 and 69 µm^2. The largest cells were monocyte, greater than erythrocyte due to their large cytoplasm. Lymphocytes presented lower size due to their small cytoplasm. The nuclear size showed similar size among the white blood cells (Table 3).

Table 3. Cell and nuclear size (µm) and area (µm^2) of blood cells from *E itajara*

	Cell Length	Cell Width	N. Length	N. Width	Cell Area
Ery	9.79±0.51	4.79±0.22	4.34±0.41	3.25±0.28	22.16±0.46
Thro	11.37±0.43	2.12±0.20	6.65±0.45	1.92±0.20	12.03±0.43
Lym	4.25±0.26	4.89±0.77	3.94±0.94	4.00±0.15	10.40±0.26
Mon	12.01±0.16	11.50±1.48	4.72±0.54	4.40±0.62	69.04±0.35
Neut	9.81±3.64	9.57±3.08	5.62±0.46	5.68±0.85	46.92±2.05
Bas	6.27±0.69	6.06±0.38	4.12±0.54	4.04±0.43	19.00±0.42

Ery-Erythrocyte, Thro-Thrombocyte, Lym-Lymphocyte, Mon-Monocyte, Neut-Neutrophil, Bas-basophil, Eos-Eosinophil, Bas-Basophil, LG-LG PAS-positive.

The multivariate analysis presented two clusters showing differences between fish with and without clinical signs (Figure 3). The components 1 and 2 (PC1 and PC2) presented 71.7% of total variance. The highest loading in the PC1 were erythrocyte (-037), lactate (-032), MCV (0.33) and MHC (0.32) (Table 4).

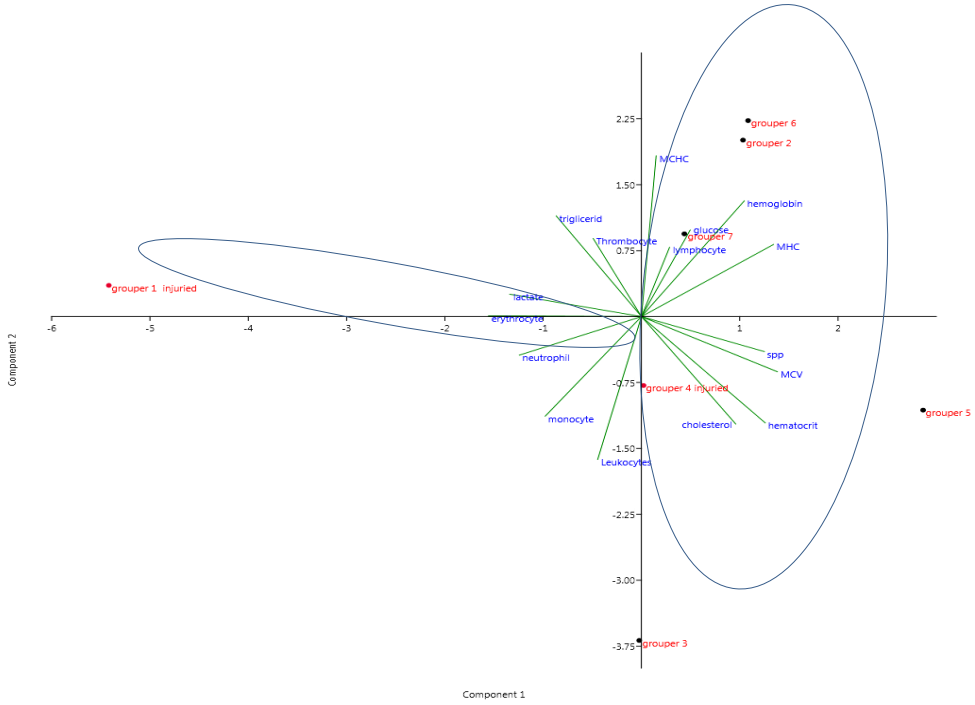

Figure 3. Erythrogram and Leukocytes on Principal Components Analysis (PCA) for *E. itajara.*

Table 4. Loadings of variables from principal components for blood of *E. itajara*

Variables	PC 1	PC 2	Variables	PC 1	PC 2
Glucose	0.11977	0.23787	MCV	0.33403	-0.15157
Triglicerid	-0.20956	0.27683	MHC	0.32466	0.19833
Cholesterol	0.23165	-0.29522	MCHC	0.036036	0.44167
Lactate	-0.32369	0.061442	Leukocytes	-0.10764	-0.3927
Serum protein	0.30191	-0.09628	Thrombocyte	-0.11857	0.21464
Erythrocyte	-0.37558	0.002257	Lymphocyte	0.06898	0.1903
Hematocrit	0.30409	-0.29218	Monocyte	-0.23641	-0.27369
Hemoglobin	0.25285	0.31812	Neutrophil	-0.30019	-0.10594

DISCUSSION

Sanitary monitoring is necessary for adaptation in captivity to adjust the handling and to prevent disease outbreaks even in species such as the goliath grouper which presents resistance to low levels of dissolved oxygen and high capacity for osmoregulation (Ranzani-paiva *et al.*, 2013; Sanches *et al.*, 2015).

Most fish presented adaptation to the captivity conditions. There were no alterations on triglyceride, cholesterol and glucose levels between fish with or without clinical signs. However, these values were lower when compared to results founded by Brum *et al.* (2018) with Nile tilapia fed with industrial diet, suggesting that with Goliath grouper a fasting period or reduction of feeding could occurr in captivity conditions such as the present work, despite the presence of preys in the tanks, such as shrimp and other fish. Blood biochemical parameters are important to measure animal stress or the influence of diets on nutritional profile (Hadi *et al.*, 2009). Thus, a feeding

management with alternative diet could improve fish health during this period.

The two fish with clinical signs due the agonistic encounters showed reduction of erythrocyte number and higher lactate levels, indicating physiological stress (Hadi *et al*., 2009), probably due to an inadequate handling management or high stocking density. Those factors caused its infection by *Vibrio parahaemolyticus*, an opportunist bacterium responsible for disease outbreaks in captivity rearing.

To mitigate these clinical signs, the location of injuries was disinfected with iodinated alcohol solution and the fish were reallocated in the separated tanks, and remained in captivity for one more week. After this period, the injuries were healed, and no agonistic encounter was reported. New blood collection was not possible because the fish would be transported during 32 hours to another laboratory.

CONCLUSION

Our results on *E. itajara* blood parameters improve knowledge about the management of this specie in captivity providing a guideline for the stress diagnostic in future studies for that species. This is the first report of hematological baseline profile for Goliath grouper *E. itajara* in captivity conditions and compared with the injured fish. The agonistic encounter showed as a stressor to *E. itajara* in tanks, for that, biggest fish (>3.5kg) should be maintained in different tanks. The feed management should be performed to improve the nutritional status in the adaptation period to those captivity conditions.

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